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TITLE: Prospective Evaluation of Hormone Replacement Therapy, Body Mass Index, Estrogen Metabolism and Breast Cancer Risk

PRINCIPAL INVESTIGATOR: Francesmary Modugno, Ph.D.

CONTRACTING ORGANIZATION: University of Pittsburg
Pittsburgh, Pennsylvania 15260

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Francesmary Modugno, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pittsburg
Pittsburgh, Pennsylvania 15260

E-Mail: fm@cs.cmu.edu

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U.S. Army Medical Research and Materiel Command
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13. ABSTRACT (Maximum 200 Words)

The objective of this project is to prospectively evaluate the extent to which BMI and estrogen metabolism are related to breast cancer associated with HRT use. We will specifically test the following hypotheses: Among postmenopausal women using HRT:

a). the risk of breast cancer is higher for women with higher serum 16-OH levels
b). *lower* BMI is associated with higher serum 16-OH levels
c). therefore: the risk of breast cancer increases with *decreasing* BMI

We will also test the relationship between serum 2-OH levels, the 2:16-OH ratio, BMI and breast cancer risk. This will be done using a nested case/control study within the observational arm of the Womens Health Initiative.

14. SUBJECT TERMS
prevention, early detection, epidemiology, molecular epidemiology, etiology; Hormone replacement therapy; body mass index; estrogen and estrogen metabolism

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INTRODUCTION:
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BODY:
In this section, we describe our accomplishments according to the Work Plan originally approved. The specific work plan tasks are listed *in italics* and our accomplishment of these tasks is shown.

Task 1: Data and Specimen Acquisition/Shipping (Months 1-6)

a. *Work with WHI Clinical Coordinating Center to select a subset of 200 cases of invasive breast cancer and 200 controls from the OS arm. Cases and controls will be frequency matched by age, race, clinical center and HRT use status.*
b. *Work with the WHI Coordinating Center to identify appropriate banked serum specimens for each subject; and arrange for the serum to be pulled, aliquotted and shipped from the repository to Immuna Care Corporation (300 microliters) .*
c. *Work with the WHI CCC to obtain demographic data on all subjects.*

We initiated a contract with the WHI CCC. We then worked closely with the WHI investigators to accomplish all of Task 1. As discussed in the next paragraphs, we accomplished items a and b; the WHI will not release demographic data on subjects until all the laboratory work is complete. In accordance with WHI policy, we applied for and received permission to conduct the analyses from the D&ACommittee (letter attached). We formed a WHI writing group and are set to begin working on the paper(s) as soon as the laboratory data are generated.

Case / Control Selection:
All centrally adjudicated cases of incident invasive breast cancer were selected as cases from the August 31, 2002 database from the WHI Observational Study. Along with being centrally adjudicated, all prospective cases also needed a completed estrogen and progesterone receptor assay data on Form 130. This left a total of 1,658 potential cases (out of an original 1,918) and 91,654 potential controls.

Potential cases and controls were excluded if they possessed any of the following characteristics:

Breast Cancer Diagnosis Less Than Five Years Past Menopause (Cases Only)
Inadequate Baseline Serum Supply
Any Locally Adjudicated Other Incident Cancers (Including Breast Cancer for Controls)
Any Self-Reported Cancer prior to or at Baseline
Reported Other/Unknown Ethnicity
Any use of the following Medications Reported at Baseline or AV-3:

Antimycobacterial Group (i.e. Rifapentine)
Imidazole-Related Anti-Fungals Group (i.e. Ketoconazole)
Androgen-Anabolic Group (i.e. Testosterone)
Adrenal Steroid Inhibitors Group (i.e. Aminoglutethimide)
Hormone Receptor Modulators Group (i.e. SERMS)
H-2 Antagonists Group (i.e. Cimetidine)
Cyclosporin Analogs (i.e. Cyclosporine)
Herbal Estrogens (i.e. dong quai)

In addition to these restrictions, potential cases and controls were also restricted to specific methods of hormone use. To meet this restrictions, participants either had to have no hormone use of any kind (as reported on Form 43 and confirmed by Baseline and AV-3 Medications) or be current users at baseline (for at least one year) of Conjugated Equine Estrogen pills, with or without Progesterone pills (as reported on Form 43). A summary of these restrictions is as follows:

Hormone Group	***Requirements***
Non HRT Users	No Use of Hormones in Any Form Reported on Form 43
	No Use of Hormones as Reported by Current Medications at Baseline & AV-3
Current Estrogen Users	Current CEE Use for at Least One Year (Form 43)
	No Non-CEE Hormone Use in the Past Year as Reported on Form 43
	Daily Estrogen Use
	No Progesterone Use within 4 years of Baseline (Form 43) and No Progesterone Use Reported on Baseline and AV-3 Current Medications
Current Estrogen + Progesterone Users	Current CEE + Progesterone Use for at Least One Year (Form 43)
	No Non-CEE Hormone Use in the Past Year as Reported on Form 43
	Daily Estrogen Use
	Continuous (Reported use of at least 25 days / month OR Prempro Every Day) or Cyclic ((Reported use between 7-16 days per month AND reported interval of use Between 5-18 days per month) OR Premphase user) Progesterone Use

Current users of CEE's were defined as those participants who listed a CEE stopping age that was the same as their age of filling out Form 43 (actual dates of use were not collected). This makes it possible for some participants listed as current users to have actually stopped using CEE's up to one year prior to their filling out of Form 43. Participants in the CEE category were not allowed to be on any estrogen or progesterone creams, shots, or implants and remain eligible. Non-hormone users were required to have never had hormone use of any kind, including shots, pills, patches, creams, and implants with either estrogen and/or progesterone.

Out of an original 1,658 cases and 91,654 controls, a total of 1,161 cases and 62,919 controls were excluded from the prospective case / control set using the above criteria, leaving a total of 497 invasive breast cancer cases (292 Current CEE Users; 205 Non HRT Users) and 28,735 controls (12,577 Current CEE Users; 16,158 Non HRT Users).

Matching criteria:
Matching is done on number of years from age at menopause to study entry (within 1 year), ethnicity, randomization clinic, CEE use (at least one year's use at baseline, never use), type of HRT (with or without Progesterone), and enrollment date (within 1 year). Ethnicity, randomization clinic, CEE use, and type of HRT (the categorical variables) were matched exactly, and the remaining continuous matching variables were selected based on criteria to minimize an overall distance measure (Bergstralh EJ, Kosanke JL. Computerized matching of cases to controls. Technical Report #56, Department of Health Sciences Research, Mayo Clinic, Rochester, MN. April 1995). Matching was done in a time forward manner to ensure that each control had at least as much control time as its matched case. For example, a case diagnosed with breast cancer two years after randomization would be matched with a control with at least two years of follow-up. SAS code is available to implement this matching scheme.

Matching summary:
A total of 497 incident cases of invasive breast cancer and 28,735 controls were put into the matching process. 468 cases were successfully matched with controls (29 unmatchable cases). A sample of 100 CEE Current User matches and 100 HRT Non-Users were selected for the study population via simple random sampling, giving a total of 200 case / control pairs.

Specific matching summaries are given in the tables below. Each row summarizes the matching performance for a specific variable or overall criteria. For example, the mean case-control absolute difference in enrollment date is 0.20 years (73.1 days), with a maximum difference of almost a year. The mean enrollment dates in the case and control groups are September 26th and September 27th, 1996, respectively. The mean case-control absolute difference in years from menopause to study entry is .14 (51.1 days). The 'overall' measurement represents the total of absolute deviations for all matching components. Thus, an overall average difference of 0.34 means that the total difference in the enrollment date plus the total difference in years from menopause to study entry averages to 0.34. The weighting equates a deviation of one year in enrollment date to a deviation of one year in the time between menopause and study entry. Ethnicity, randomization clinic, HRT use, and HRT type are matched exactly for all subjects.

Balance on each covariate individually and overall is sufficient.

Matching Factor	Sum (weighted) of Absolute Differences	Cases	Controls
	Mean (min, max)	Mean	Mean
Overall	0.34 (0, 1.56)	-	-
Ethnicity	0	-	-
Randomization Clinic	0	-	-
HRT Use (Current or Never)	0	50% Current	
HRT Type (with or without Prog.)	0	60% PERT	
Enrollment Date (years)	0.20 (0 , 0.99)	9 / 26 / 96	9 / 27 / 96
Menopause to Study Entry (years)	0.14 (0 , 1.00)	15.51	15.52

Task 2: Perform Laboratory Assays (Months 4-12)

a. Assay samples to measure metabolite levels

b. Retest 40 specimens to validate laboratory results

We have arranged for shipping of the WHI serum to Immunacare Corporation. We are behind schedule here because we were required to follow WHI protocol. However, we have made arrangements with Immuncare Corp, such that the data will be completed within 1 month after serum is shipped. The estimated date of shipment is July, 2003. Thus, we anticipate data to be fully available by September, 2003. In the interim, we have devised a set of quality control procedures in collaboration with Dr. Klug of Immunacare corporation.

Problems encountered and measures taken: NONE

KEY RESEARCH ACCOMPLISHMENTS: NONE

REPORTABLE OUTCOMES: NONE

CONCLUSIONS:

At this time, we are in the process of gathering the data for this study. We anticipate all the data will be generated in the first half of year 2. We will then work with the WHI CCC to analyze the data and report the results.

APPENDICES:

Approval letters from WHI for analyzing data

FRED HUTCHINSON CANCER RESEARCH CENTER

WOMEN'S HEALTH INITIATIVE

WHI Clinical Coordinating Center

MEMORANDUM

Date: September 10, 2002

To: Francesmary Modugno
Pittsburgh

From: Sundara Murphy
P&P Program Assistant
Phone: 206-667-4987
Fax: 206-667-4142

Subject: Manuscript #209 – *Estrogen Metabolism, Body Mass Index, Hormone Replacement Therapy and Post-menopausal Breast Cancer Risk*

Congratulations! The P&P Committee reviewed and approved this paper proposal. They have the following comments:

1. You won't be able to classify by one or two ovaries because WHI does not collect this data.
2. Antigen use should be taken into consideration.
3. Thyroid dysfunction may need to be considered in the analysis.
4. You will need to be especially careful to not contradict CT results.

The Project Office has approved this manuscript proposal. See the enclosed comments for more details. Please contact Barbara Howard, P&P Committee chair, if you have any questions.

As this is a WHI OS Blood Study, you will need to follow P&P authorship policy, which states that the writing group may have 4 co-authors from the study and 4 study-wide WHI investigators as co-authors. WHI Policy states that writing groups are limited to 8 authors. I will open this paper up for writing group nominations within WHI and contact you when that process is complete. If there are more than 4 investigators from this study interested in authorship of this paper, please choose the 4 (including yourself) and let the others know they may nominate themselves to the writing group. If there are less than 4 study-wide WHI investigators nominated, you may add investigators so there is a total of 8

cc: Barbara Howard, central files

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1100 Fairview Avenue North, MP – 1002
P.O. Box 19024
Seattle, WA 98109—1024
Ph.: 206-667-6883
Fx.: 206-667-4142

writing group members. Please let me know the four individuals from the ancillary study who will be participating on the writing group.

WHI Manuscript Proposal Review

Reviewer:

Title: **Ms 209 – Estrogen Metabolism, Body Mass Index, Hormone Replacement Therapy and Post-menopausal Breast Cancer Risk**

Convener: Francesmary Modugno

Please address each of the criteria below and type in your responses in the spaces provided below:

Possible overlaps with other P&P approved papers:
None.

Scientific Merit:
Excellent. The mechanistic papers add a great deal to observational studies, and it seems as though this particular analysis will be very important.

Analytical issues + Interpretation and discussion issues:

With regard to variables, it will be difficult to classify participants by removal of one or two ovaries. Also, some women may have used antigens in addition to estrogens and progestins. I don't know whether the numbers are sufficient, but it might be something for the investigators to consider. Other related conditions such as thyroid dysfunction, etc. may need to be considered in the analyses. Also, it may be possible to use the fat measurements from the DEXA centers to provide additional information as the authors note that body fat is the key factor rather than body weight.

Policy Issues:
Need to make sure that whatever is written is not contradictory to trial results. Efforts should be made to view the interpretation in light of the clinical trial findings. Also, wasn't sure where they are going to get the blood to do these analyses for serum levels of estrones.

Reviewer recommendation:

_____ **Approval**

__xx__ **Approval with response to comments.**

_____ **Revise and Resubmit**

_____ **Disapproval**

Proposal No. 209

DUE 8/26/02

WHI PROPOSAL MANUSCRIPT REVIEW

Title: Estrogen Metabolism, Body Mass Index, HRT and Post-Menopausal Breast Cancer Risk

Convener (non-NIH): F Modugno, L Kuller, K Kip

Convener (NIH):

Review:

Analytical Issues (including approach and presentation):

None

Policy Issues:

Race-ethnicity should correspond to P&P guidelines (eg Asian/Pacific Islander, American Indian, etc)

Consider avoiding the term "HRT" (FDA is moving to remove the connotation of "replacement" from postmenopausal hormone therapy).

Recommendation:

__X__ **Approval** _____ **Approval with Changes** _____ **Disapproval**

Recommended Changes:

Signature: Jacques E. Rossouw **Date:** 8/22/02

FRED HUTCHINSON CANCER RESEARCH CENTER

WOMEN'S HEALTH INITIATIVE

WHI Clinical Coordinating Center

MEMORANDUM

DATE: June 17, 2003

TO: Francesmary Modugno
Pittsburgh

FROM: Sundara Murphy
P&P Program Assistant

RE: Ms 209 - Estrogen Metabolism, Body Mass Index, Hormone Replacement Therapy and Post-menopausal Breast Cancer Risk

On behalf of the WHI Publications and Presentations Committee, I would like to congratulate you on your appointment to Chair of the manuscript referenced above.

Our mutual goal is the orderly and expeditious publication of this WHI manuscript. Towards this end, we look forward to working with you and members of your Writing Group to facilitate preparation of the first draft.

As Chair of a writing group, you assume responsibility for coordinating analysis and writing efforts and ensuring that the manuscript is completed according to the pre-determined production timeline. The P&P Committee will monitor your progress and will offer assistance, should you encounter any problems in adhering to the production schedule. The enclosed attachment, entitled "WHI Responsibilities of Writing Group Chair and Writing Group Members," details the responsibilities involved in preparing the approved paper. Please review these guidelines carefully. Also enclosed, please find a copy of "Analysis Process and Analysis Plan Guidelines" and "Guidelines for WHI Writing Groups". A copy of this letter and the enclosed guidelines have been sent to members of your Writing Group so that they too will be aware of their responsibilities.

High quality and credible publications represent the ultimate goal of ambitious projects such as the WHI. As scientists and study investigators, we have a responsibility to disseminate relevant information to the scientific community in a timely manner. Your efforts support these goals.

C:\fmm-mail\attach\Ms209wg.doc

1100 Fairview Ave. N. MP 1002, PO Box 19024
Seattle, Washington 98109-1024
206-667-6883 FAX 206-667-4142

Your Writing Group consists of the following individuals:

Name	Clinic Center	Email address	Phone number	Fax number
Tom Rohan	New York	rohan@aecom.yu.edu	718-430 3355	718-430 8653
Rowan Chlebowski	Torrance	rchlebow@whi.org	310-222-2217	310-320-2564
Norm Lasser	Newark	nlasser@whi.org	973-972-5544	973-972-7646
Marcia Stefanick	Stanford	stefanick@stanford.edu	650-725-5041	650-723-7018
Francesmary Modugno	Pittsburgh	fm@cs.cmu.edu	412-383-2601	412-383-1121
Lew Kuller	Pittsburgh	KullerL@edc.pitt.edu	412-383-1895	412-383-1896
Kevin Kip Barb Cochrane	CCC	barbc@whi.org	206-667-6814	206-667-4142